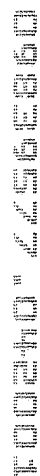
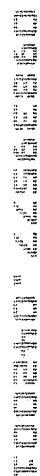


[illegible][illegible]

- [illegible]

[illegible][illegible][illegible][illegible]

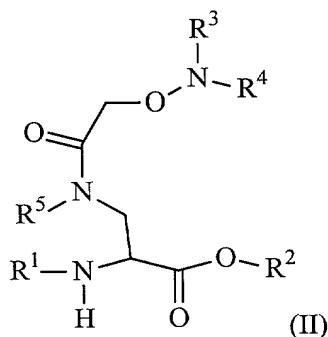
- [illegible]

[illegible][illegible]

- [illegible]

[illegible][illegible]

- [illegible]



wherein:

- 5 R¹ is hydrogen or an amino protecting group;

 R² is hydrogen or a carboxy protecting group;

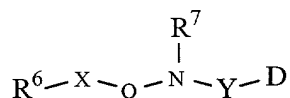
 R³ is (C₁-C₆)alkyl;

 R⁴ is hydrogen, or an amino protecting group; and

 R⁵ is hydrogen.
- 10 4. The compound of claim 3 wherein R⁴ is 2-chlorobenzyloxycarbonyl.
- 5. The compound of claim 1 which is α-benzyloxycarbonyl-β-[N-(2-chlorobenzyloxycarbonyl)-N-methylaminoxyacetyl]-α,β-diaminopropionic acid.
- 15 6. A peptide comprising a backbone and one or more aminoxy groups; provided the peptide is not glutathione; and provided the peptide has at least one aminoxy group that is not part of a group H₂N-O-CH₂-C(=O)- positioned at the N-terminus of the peptide or that is

20 not part of a group -C=N-O-CH₂-C(=O)- that is in the backbone.
- 7. A peptide comprising a backbone and one or more secondary aminoxy groups; provided the peptide has at least one aminoxy group that is not part of

25 an oxime (C=N-O-) in the backbone.
- 8. A peptide conjugate of formula (III):



(III)

wherein

R^6 is a peptide or polypeptide;

X is a direct bond or a linking group;

R^7 is hydrogen, $(\text{C}_1\text{-C}_6)$ alkyl, an amino protecting group, or a radical

5 comprising one or more aminooxy groups;

Y is a direct bond or a linking group; and

D is a functional molecule.

9. The peptide conjugate of claim 8 wherein R^6 is an antibody.

10

10. The peptide conjugate of claim 8 wherein R^6 comprises about 2 to about 1000 amino acids.

11. The peptide conjugate of claim 8 wherein R^6 comprises about 5 to
15 about 500 amino acids.

12. The peptide conjugate of claim 8 wherein R^6 comprises about 10 to about 100 amino acids.

20 13. The peptide conjugate of claim 8 wherein X is about 5 angstroms to about 100 angstroms in length.

14. The peptide conjugate of claim 8 wherein X is about 5 angstroms to about 25 angstroms in length.

25

15. The peptide conjugate of claim 8 wherein X is $-\text{R}_a-\text{C}(=\text{O})-\text{NH}-\text{R}_b-$ wherein each of R_a and R_b is independently $(\text{C}_1\text{-C}_6)$ alkylene.

16. The peptide conjugate of claim 15 wherein each of R_a and R_b is
30 methylene $(-\text{CH}_2-)$.

17. The peptide conjugate of claim 8 wherein R⁷ is hydrogen.
18. The peptide conjugate of claim 8 wherein R⁷ is (C₁-C₆)alkyl.
19. The peptide conjugate of claim 8 wherein R⁷ is methyl.
20. The peptide conjugate of claim 8 wherein R⁷ is a radical comprising one or more aminoxy groups.
21. The peptide conjugate of claim 8 wherein Y is about 5 angstroms to about 100 angstroms in length.
22. The peptide conjugate of claim 8 wherein Y is about 5 angstroms to about 25 angstroms in length.
23. The peptide conjugate of claim 8 wherein Y is (C₁-C₆)alkylene.
24. The peptide conjugate of claim 8 wherein Y is methylene (-CH₂-).
25. The peptide conjugate of claim 8 wherein D is a biophysical probe, a peptide, a polynucleotide, or a therapeutic agent.
26. The peptide conjugate of claim 8 wherein D is a cross-linking group or a caged response modifier.
27. The peptide conjugate of claim 8 wherein D is a biophysical probe.
28. The peptide of claim 27 wherein the biophysical probe is a fluorescent group, a phosphorescent group, a nucleic acid indicator, an ESR probe, a responsive sensor, a caged sensor, or a dye that is sensitive to pH change, ligand binding, or other environmental aspects.
29. The peptide of claim 8 wherein D is a peptide.

30. The peptide of claim 8 wherein D is a polynucleotide.
31. The peptide of claim 30 wherein the polynucleotide is DNA
- 5 32. The peptide of claim 30 wherein the polynucleotide is RNA
33. The peptide of claim 8 wherein D is a therapeutic agent.
- 10 34. The peptide of claim 8 wherein D is an Alexa dye, a solvatochromic dye, an electrochromatic dye, or a dye that is sensitive to pH change, ligand binding, or other environmental aspects.
- 15 35. The peptide of claim 8 wherein D is Alexa-532, Hydroxycoumarin, Aminocoumarin, Methoxycoumarin, Amino methylcoumarin, Cascade Blue, Lucifer Yellow, NBD, P-Phycoerythrin, R-Phycoerythrin, (PE), PE-Cy5 conjugates, PE-Cy7 conjugates, Red 613, Fluorescein, BODIPY-FL, BODIPY TR, BODIPY TMR, Cy3, TRITC, X-Rhodamine, Lissamine Rhodamine B, PerCP, Texas Red, Cy5, Cy7, Allophycocyanin (APC), TruRed, APC-Cy7
- 20 conjugates, Oregon Green, Tetramethylrhodamine, Dansyl, Indo-1, Fura-2, FM 1-43, DilC18(3), Carboxy-SNARF-1, NBD, Indo-1, Fluo-3, DCFH, DHR, SNARF, or Monochlorobimane, Calcein.
36. The peptide of claim 8 wherein D is YOYO-1, Propidium Iodide,
- 25 Hoechst 33342, DAPI, Hoechst 33258, SYTOX Blue, Chromomycin A3, Mithramycin, SYTOX Green, SYTX Orange, Ethidium Bromide, 7-AAD, Acridine Orange, TOTO-1, TO-PRO-1, Thiazole Orange, Propidium Iodide, TOTO-3, TO-PRO-3, or LDS 751.
- 30 37. The peptide of claim 8 wherein:
 R^6 is (SEQ ID NO. 1);
X is $R_a-C(=O)CH(NH_2)CH_2N(H)C(=O)CH_2-R_b$; wherein R_a is a direct bond to the amino terminus of R^6 and wherein R_b is a direct bond to the oxygen of the aminooxy group of formula (III);

R⁷ is methyl;
Y is a direct bond; and
D is Alexa-532.

5 38. A method for preparing a peptide conjugate comprising a peptide
linked to a functional molecule, comprising reacting a peptide having one or
more secondary aminooxy groups with a corresponding functional molecule
having an electrophilic moiety that is reactive with the aminooxy group(s), to
provide the peptide conjugate.

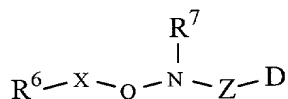
10

39. A method for preparing a peptide conjugate comprising a peptide
linked to a functional molecule, comprising reacting a peptide having one or
more aminooxy groups with a corresponding functional molecule having an
electrophilic moiety that is reactive with the aminooxy group(s), to provide the
15 peptide conjugate; provided that the functional molecule is not a peptide.

40. A method for preparing a peptide conjugate comprising a peptide
linked to a functional molecule, comprising reacting a peptide having one or
more aminooxy groups with a corresponding functional molecule having an
20 electrophilic moiety that is reactive with the aminooxy group(s), to provide the
peptide conjugate; provided that the peptide and the functional molecule are not
attached through an oxime (C=N-O-) linkage.

41. A method for preparing a peptide conjugate comprising a functional
25 molecule linked to a peptide having a backbone, comprising reacting a peptide
having one or more aminooxy groups with a corresponding functional molecule
having an electrophilic moiety that is reactive with the aminooxy group(s), to
provide the peptide conjugate; provided that when the functional molecule is a
second peptide, the functional molecule and the first peptide are not linked
30 through a -C=N-O-CH₂-C(=O)- linkage in the backbone of the first peptide.

42. A peptide conjugate of formula (IV):



(IV)

wherein

R⁶ is a peptide, polypeptide or antibody;

X is a direct bond or a linking group;

R⁷ is hydrogen, (C₁-C₆)alkyl, an amino protecting group, or a radical

5 comprising one or more aminooxy groups;

Z is a linking group, or Z is a direct single bond or a double bond
between N and D; and

D is a functional molecule;

provided that when D is a peptide, N and D are not linked through a -
10 C=N-O-CH₂-C(=O)- linkage in the backbone of the peptide conjugate.

43. The peptide conjugate of claim 42 wherein R⁶ is an antibody.

44. A method of identifying an optimal position for placement of a
functional molecule on a peptide having a peptide backbone and a known
15 activity, which comprises making a series of peptide conjugates, each peptide
conjugate having the same amino acid sequence and the same functional
molecule, wherein the functional molecule is linked at a different location along
the backbone of every peptide conjugate in the series, and observing which
functional group location does not substantially interfere with the known activity
20 of the peptide.

45. The method of claim 44 wherein each peptide of said series of peptide
conjugates comprises the peptide conjugate of claim 8 or 42.

25 46. A method of identifying an optimal position for placement of a
functional molecule in a polypeptide having a known activity and an identified
peptide segment for attachment of the functional molecule, which comprises:
a) making a series of peptide conjugates, each peptide conjugate
having the amino acid sequence of the identified peptide segment
30 and the same functional molecule, wherein the functional molecule

is linked at a different location along the backbone of every peptide conjugate in the series;

- b) placing a peptide conjugate within, or at the end of, each polypeptide of a series of polypeptides to create a series of polypeptide conjugates each having the functional group at a different location; and
- c) observing which functional group location does not substantially interfere with the known activity of the polypeptide.

47. The method of claim 46 wherein each peptide of said series of peptides comprises the peptide conjugate of claim 8 or 42.

48. The method of claim 46 wherein each peptide conjugate is placed within, or at the end of, each polypeptide by natural chemical ligation, intein-mediated protein ligation or chemical ligation.

49. A method of identifying an optimal position for placement of an environmentally-sensitive functional molecule on a peptide biosensor having a backbone, which comprises making a series of peptide conjugates, each peptide conjugate having the same amino acid sequence and the same functional molecule, wherein the functional molecule is at a different location along the backbone of every peptide conjugate in the series, and observing which functional group location provides the strongest signal change in response to an environmental change in the peptide conjugate.

50. The method of claim 49 wherein each peptide conjugate of said series of peptide conjugates comprises the peptide conjugate of claim 8 or 42.

51. The method of claim 49 wherein each peptide conjugate is part of a polypeptide, antibody, antibody fragment, antibody fragment with linked chains or antibody with linked chains.

52. The method of claim 49 wherein said signal change is a change in phosphorescence, fluorescence emission intensity, fluorescence lifetime, fluorescence excitation wavelength or fluorescence emission wavelength.

53. The method of claim 49 wherein said environmental change in said peptide biosensor is interaction with a target.

5 54. The method of claim 53 which further comprises observing the binding affinity of each peptide conjugate for target or the binding selectivity of each peptide conjugate for target.

55. A method of identifying an optimal position for placement of an
10 environmentally-sensitive functional molecule in a polypeptide having a known activity and an identified peptide segment for attachment of the functional molecule, which comprises:

- 15 a) making a series of peptide conjugates, each peptide conjugate having the amino acid sequence of the identified peptide segment and the same environmentally-sensitive functional molecule, wherein the environmentally-sensitive functional molecule is linked at a different location along the backbone of every peptide conjugate in the series;
- 20 b) placing a peptide conjugate within, or at the end of, each polypeptide of a series of polypeptides to create a series of polypeptide conjugates each having the environmentally-sensitive functional group at a different location; and
- 25 c) observing which functional group location provides the strongest signal change in response to an environmental change in the polypeptide conjugate.

56. The method of claim 55 wherein each peptide conjugate of said series of peptide conjugates comprises the peptide conjugate of claim 8 or 42.

30 57. The method of claim 55 wherein each peptide conjugate is placed within, or at the end of, each polypeptide by natural chemical ligation, intein-mediated protein ligation or chemical ligation.

58. The method of claim 55 wherein said signal change is a change in phosphorescence, fluorescence emission intensity, fluorescence lifetime, fluorescence excitation wavelength or fluorescence emission wavelength.
- 5 59 The method of claim 55 wherein said environmental change in said protein conjugate is interaction with a target or a change in activation state of a target.
60. The method of claim 59 which further comprises observing the binding
10 affinity of each peptide conjugate for target or the binding selectivity of each peptide conjugate for target.
61. A polypeptide biosensor which comprises the peptide conjugate of claim 8 or 42.
- 15 62. The polypeptide biosensor of claim 61 which comprises a p21 activated kinase peptide capable of binding Rac or a Wiscott-Aldrich Syndrome Protein peptide capable of binding cdc42.
- 20 63. A polypeptide biosensor comprising a polypeptide capable of binding a GTP-activated Rho GTPase protein, wherein said polypeptide is operatively linked to a fluorescent dye which is capable of fluorescence resonance energy transfer with a fluorescence dye on said GTP-activated Rho GTPase protein.
- 25 64. The polypeptide biosensor of claim 63 wherein said Rho GTPase protein is Rac or cdc42.
65. The polypeptide biosensor of claim 63 wherein said polypeptide comprises the protein binding domain of p21 activated kinase 1.
- 30 66. The protein biosensor of claim 63 wherein said fluorescence dye on said GTP-activated Rho GTPase protein is Green Fluorescence Protein (GFP), Cyan Fluorescence Protein(CFP), Red Fluorescence Protein (RFP) or enhanced GFP (EGFP).

67. A fusion protein comprising a biologically active Rho GTPase protein domain operatively linked to a fluorescent protein via the peptide conjugate of claim 8 or 42, wherein said Rho GTPase protein domain is capable of binding
5 GTP and forming an activated GTPase:GTP complex.
68. The fusion protein of claim 67 wherein said Rho GTPase protein domain is derived from a Rac, Rho or cdc42 protein.
- 10 69. The fusion protein of claim 67 wherein said fluorescence protein is green fluorescence protein, cyan fluorescence protein, red fluorescence protein, yellow fluorescence protein, enhanced green fluorescence protein or enhanced yellow fluorescence protein.
- 15 70. A nucleic acid encoding the fusion protein of claim 67.
71. An expression vector capable of expressing the fusion protein encoded by the nucleic acid of claim 67.
- 20 72. A cell comprising the expression vector of claim 71.
73. A method for detecting GTP activation of a Rho GTPase protein in a live cell comprising:
- 25 a) introducing a Rho GTPase protein to said cell, wherein said Rho GTPase protein is operatively linked to a fluorescence dye capable of undergoing fluorescence resonance energy transfer;
- b) introducing a polypeptide biosensor into said cell, wherein said polypeptide biosensor comprises a polypeptide capable of binding
30 a GTP-activated Rho GTPase protein, and wherein said polypeptide is operatively linked to a fluorescent dye which can undergo fluorescence resonance energy transfer with said fluorescence dye on said GTP-activated Rho GTPase protein when said polypeptide biosensor interacts with said GTP-activated Rho GTPase; and

- c) observing fluorescence emissions from said polypeptide biosensor within said live cell.

74. The method of claim 73 which further comprises observing
5 fluorescence emissions from said fluorescence dye on said GTP-activated Rho GTPase.

75. The method of claim 73 wherein said polypeptide biosensor comprises the peptide conjugate of claim 8 or 42.

10

76. A method for detecting GTP activation of a Rho GTPase protein, which comprises:

- a) contacting a polypeptide biosensor with a test substance, wherein said polypeptide biosensor comprises a polypeptide capable of
15 binding a GTP-activated Rho GTPase protein, and wherein said polypeptide is operatively linked to an environmentally sensitive dye; and

b) observing a signal from said polypeptide;
wherein said environmentally sensitive dye will emit a signal of a different
20 lifetime, intensity or wavelength when said polypeptide biosensor is bound to said GTP-activated Rho GTPase protein than when said polypeptide biosensor is not bound.

77. A method for detecting GTP activation of a Rho GTPase protein in a live
25 cell comprising:

- a) introducing a polypeptide biosensor into said cell, wherein said polypeptide biosensor comprises a polypeptide capable of binding a GTP-activated Rho GTPase protein, and wherein said polypeptide is operatively linked to an environmentally sensitive dye; and
30 b) observing a signal from said environmentally sensitive dye within said live cell;

wherein said environmentally sensitive dye will emit a signal of a different lifetime, intensity or wavelength when said polypeptide biosensor is bound to

said GTP-activated Rho GTPase protein than when said polypeptide biosensor is not bound.

78. The method of claim 76 or 77 wherein said polypeptide biosensor
5 comprises the peptide conjugate of claim 8 or 42.

79. The method of claim 76 or 77 wherein said polypeptide biosensor
comprises the protein binding domain of p21 protein kinase or the protein
binding domain of Wiscott Aldrich syndrome protein.

10

80. The method of claim 76 or 77 wherein said Rho GTPase protein
domain is derived from a Rac, Rho or cdc42 protein.

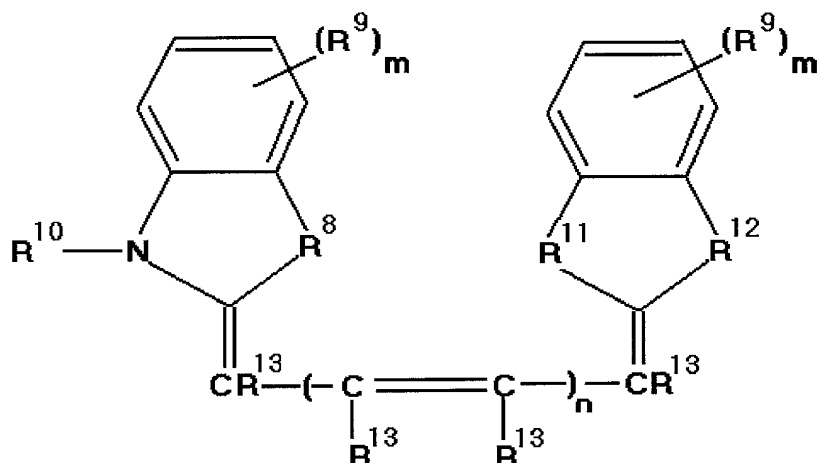
81. The method of claim 76 or 77 which further comprises quantifying the
15 amount of GTP-activated Rho GTPase protein.

82. A method for detecting binding of an antibody to an antigen which
comprises reacting an antibody comprising the peptide conjugate of claim 8 or
42 with an antigen and detecting an antibody-antigen complex.

20

83. A method for detecting binding of an antigen to an antibody which
comprises reacting an antigen comprising the peptide conjugate of claim 8 or 42
with an antibody and detecting an antibody-antigen complex.

84. A fluorescent compound of the formula:

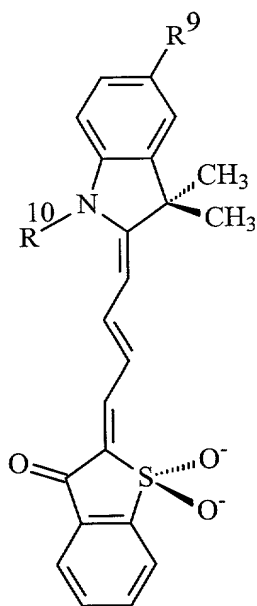


wherein:

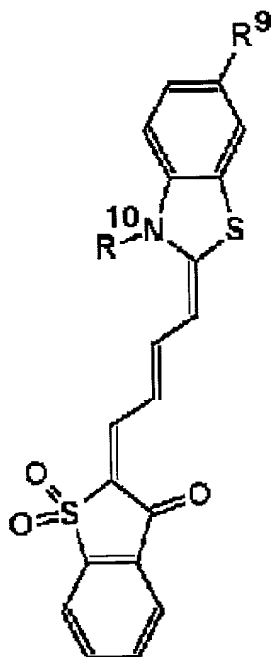
- 5 each m is separately an integer ranging from 1-3;
n is an integer ranging from 0 to 5;
R⁸, R¹¹ and R¹² are separately CO, SO₂, C=C(CN)₂, S, O or C(CH₃)₂ ;
each R¹³ is alkyl, branched alkyl or heterocyclic ring derivatized
10 with charged groups to enhance water solubility and enhance photostability;
each R⁹ and R¹⁰ is separately an alkyl chain derivatized with charged groups to enhance water solubility or with reactive groups for conjugation to other molecules.
- 15 85. The compound of claim 84 wherein each R⁹ and R¹⁰ is separately sulfonate, amide or ether.
86. The compound of claim 84 wherein each R⁹ and R¹⁰ is separately
-NH-(C=O)-CH₂-halide, amine, maleimide, -N=C=O, -N=C=S, acyl halide,
20 succinimidyl ester, sulfosuccinimidyl ester, sulfonyl halide, sulfonyl azide, alcohol, thiol, semicarbazide, hydrazine or hydroxylamine.
87. The compound of claim 84 wherein each R⁹ and R¹⁰ is separately carboxylic acid, alkali or alkaline earth metal salt of carboxylic acid, carboxylic

acid activated by carbodiimide, acyl chloride, succinimidyl, sulfosuccinimidyl ester or COOR-x, wherein x is phenol or naphthol further substituted by at least one strong electron withdrawing group.

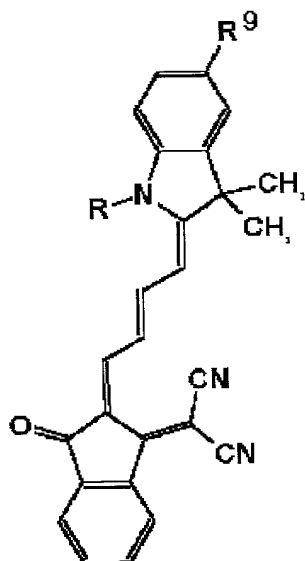
- 5 88. The compound of claim 84 having the formula:



89. The compound of claim 84 having the formula:



90. The compound of claim 84 having the formula:



5

91. A peptide biosensor comprising the compound of claim 84.

10

92. A protein, polypeptide, peptide, antibody, antibody fragment or nucleic acid linked to the compound of claim 84.

09097 0400
T00270 " 2568860